Results: In contrast to our original hypothesis, we found that blood flow recovery was severely impaired in RP105−/− mice. Immunohistochemistry showed that both arteriogenesis and angiogenesis were reduced in these mice compared to wildtype animals. However, both in vivo and ex vivo analyses showed that circulatory pro-arteriogenic Ly6Chi monocytes were more readily activated in RP105−/− mice. FACS analyses in blood and tissue samples from wildtype mice showed that Ly6Chi monocytes migrate to the affected muscle tissues following induction of hind limb ischemia. Although Ly6Chi monocytes were more readily activated in RP105−/− mice, migration into the ischemic tissues was severely hampered and instead, Ly6Chi monocytes accumulated in their storage compartments, bone marrow and spleen, of RP105−/− mice. In vitro studies showed that activation of monocytes with LPS reduces their migratory ability, indicating that a timely, well-regulated activation of monocytes is crucial for effective neovascularisation.
Conclusion: The lack of TLR4 regulation in RP105−/− mice results in an unrestrained inflammatory response, leading to systemic monocyte over-activation. Monocyte activation reduces their migratory ability and thus, this premature, systemic activation of pro-inflammatory Ly6Chi monocytes results in reduced infiltration of Ly6Chi monocytes in affected tissues after ischemia and consequently in reduced arteriogenesis and angiogenesis. Therefore, a tightly regulated inflammatory response is crucial for effective neovascularisation and blood flow recovery after ischemia.

Preclinical Small Animal Model for Studying Ischemia-reperfusion Injury of the Spinal Cord After Crossclamping of the Aorta and the Beneficial Effect of EPO on the Neuronal Function

F. Simon, A. Oberhuber, P. Erhardt, H. Schelzig

Department of Vascular and Endovascular Surgery, University of Düsseldorf, Düsseldorf, Germany

Introduction: Ischemia and reperfusion (I/R) of the spinal cord are central problems of aortic surgery procedures e.g. aneurysm repair. In a large animal model we showed in the past that erythropoietin (EPO) attenuates the morphological signs of spinal cord I/R-injury and improves neurological function, but observation time was only 10 hours short. This small animal model with optimized clamping time evaluates the benefit of perioperative i.v. EPO administration and gives answer to the question of the clinical outcome up to 4 days.
Methods: To evaluate the optimal clamping time 36 New Zealand White Rabbits were used. Unlike other animal species rabbits have a pure segmental blood supply for the spinal cord, therefore reproducible paraplegia can be performed by infrarenal clamping alone. The ischemic times ranged from 15 over 17 to 20, 22 and 25 min to define best fitting clamping time. Afterwards rabbits received either vehicle (control, $n = 10$) or EPO ($n = 10$; 5000 IU/kg) over the last 30 min before clamping and during the first 30 minutes of reperfusion. Intraoperatively blood pressure (invasive), heart rate, oxygen saturation and temperature were recorded. In addition, blood samples were taken before and after aortic clamping for studies on senescence and apoptosis parameters. Clinical neurological examinations were performed using a modified Tarlov score every twelve hours. After 96 h the entire spinal cord was harvested for histological examination.
Results: After 96 h postoperative observation period animals of the 15 min clamping group showed a Tarlov score of 3.65 ± 1.55; 17 min 4 ± 1.75; 20 min 1.5 ± 2.22; 22 min 0 ± 1.59; 25 min 0 ± 0. Histological and clinical findings were significantly correlated, $p = 0.007$. Therefore a clamping time of 22 minutes was defined for further experiments. The following study groups consisted of control animals (NaCl; $n = 10$) and EPO treated animals ($n = 10$) challenged with 22 minutes aortic clamping. While the control animals showed no neuronal function at all (median = 0), the EPO group standed out with improved spinal cord function (median = 4,25) after 36 hours of reperfusion. This benefit was lost again after 96 hours of reperfusion (median = 0).
Conclusion: This model is an ideal setup for therapeutic studies on ischemia and reperfusion injury (I/R) after aortic crossclamping. EPO showed impressive benefits in ischemic neuronal tissue by improving spinal cord function.

Shear Stress Induces Vasoprotective Gene Upregulation in Pericytes

C. Schrimpf, K. Tim, U. Böer, M. Klingenberg, O. Teebken, M. Wilhelmi

Division of Vascular and Endovascular Surgery, Department of Cardiothoracic Transplantation and Vascular Surgery, Hannover Medical School, Hannover, Germany

Introduction: Fibrosis is an initial step in the development of atherosclerotic lesions, hallmarked by myofibroblasts entering the tunica media and forming a fibrous cap. Vasa vasorum in the adventitia consist of endothelial cells, surrounded by pericytes. Pericytes can be regarded as cells with stem cell like properties and the capacity to differentiate into myofibroblasts. Although it is known that shear stress induces atherosclerotic lesions, nothing is known about its impact on pericytes. Herein, we investigate the effect of shear stress on pericytes and focus on differentiation into myofibroblasts.
Methods: Primary human pericytes (PCs) were isolated from donor fatty tissue and cultured in DMEM + 10% FCS. Primary cells were characterised by immunofluorescent (IF) staining (NG2, CD90, CD105, CD44, CD45, CD73, CD146, CD31, and PDGFR-beta). Endothelial cells (HUVEC) or PC were seeded into flow chambers and subjected to laminar flow at low 10 dyn, high 30 dyn and no shear stress rates for 48 h ($n = 3$/group). RNA was extracted and analysed by qPCR for tissue inhibitor of metalloproteinase 3, versican

and genes known to be regulated in pericyte — myofibroblast transition. In addition, IF staining for cytoskeletal f-actin and VE-cadherin in HUVECS was performed.
Results: Characterisation of PCs showed positivity for CD44, Cd90, PDGFR-beta, CD105, NG2, and negativity for CD31, CD45, CD146 and CD73. HUVEC subjected to low and high shear stress aligned and elongated in flow direction. PCs subjected to shear stress, revealed an opposite behavior to HUVEC, aligning almost perpendicular to flow. qPCR analysis of PCs and HUVEC with no, 10 or 30 dyn shear stress revealed that endothelial cells upregulated the extracellular matrix protein versican (2 fold increase of normalized gene expression to GAPDH) and its protease ADAMTS1 (4 fold increase), while TIMP3, a tissue inhibitor of matrix metalloproteinase was upregulated under high shear stress in pericytes (3 fold upregulation). In addition, plasma was upregulated in HUVEC but not PC.
Conclusion: Shear stress induces extracellular matrix turnover and in pericytes leads to upregulation of proteases known to stabilise the vascular wall. Pericytes in contrast to endothelial cells align perpendicular to flow direction. Co-culture experiments with pericytes and ECs under flow are planned to verify monoculture results.

HIF-1α Expression Precedes Ephrin-B2 Expression During AVF Maturation

N. Sadaghianloo, C. Protack, K. Yamamoto, M. Tsuneki, S. Declemy, R. Hassen-Khodja, J. Madri, A. Dardik

Department of Surgery, Yale University, USA

Introduction: Arteriovenous fistulae (AVF) continue to show poor clinical results, with 50% 1-year patency. To understand AVF maturation, we previously reported a novel mouse AVF model recapitulating human AVF maturation. In order to determine if AVF have increased arterial identity, we examined Ephrin-B2 expression, an embryonic determinant of arteries. Since AVF creation may be associated with surgical injury and generation of reactive oxygen species, we also examined whether HIF-1α is expressed as well as its temporal relationship to Ephrin-B2 expression.
Methods: Aortocaval fistulae were created in C57Bl/6 mice, and sham-operated mice were controls. Specimens of AVF or inferior vena cava were explanted up to day 42. Analysis was performed with Amplex Red for extracellular H2O2, and qPCR, immunohistochemistry, and Western blotting for Ephrin-B2 and HIF-1α.
Results: AVF released more extracellular H2O2 compared to veins ($n = 3$; $p = 0.007$). AVF expressed increased numbers of Ephrin-B2 transcripts between days 7 and 21 post-operatively ($n = 8$; $p < 0.05$, ANOVA), and increased numbers of HIF-1α transcripts between days 3 and 21 ($n = 8$; $p < 0.05$, ANOVA). Western blot showed increased Ephrin-B2 ($n = 3$; $p = 0.036$) and HIF-1α ($n = 3$; $p = 0.049$) protein density compared to veins (postoperative day 3). Immunohistochemistry showed increased Ephrin-B2 and HIF-1α immunoreactivity in the AVF endothelium ($n = 2$; day 3).
Conclusion: AVF have increased expression of both Ephrin-B2 and HIF-1α during early maturation. HIF-1α expression temporally precedes Ephrin-B2 expression, suggesting that HIF-1α may induce Ephrin-B2. These results suggest that clinical strategies to improve AVF outcomes could target the oxidative stress pathway.

Hyperglycaemia Exaggerates Ischaemia Induced Tissue Damage in Dermal Fibroblasts Through MyD88 Dependant Toll-like Receptor 4 Activation

M.J. Portou, R. Yu, X. Shi-wen, D. Abraham, G. Hamilton, D. Baker, J. Tsui

Royal Free Vascular, UCL Department of Surgery and Interventional Sciences, 9th Floor, Royal Free Hospital NHS Foundation Trust, Pond Street, London NW3 2QG, UK

Introduction: Diabetic foot ulceration is a common and challenging complication of diabetes, and leads to a 20 times greater risk of major amputation compared to non-diabetics. There is increasing recognition of diabetes as a pro-inflammatory condition and evidence that toll-like receptor 4 (TLR4) activation is involved in its systemic pathogenesis and the subsequent impairment of wound healing, however the mechanism remains unclear. We aim to study the effect of simulated diabetic-ischaemic conditions on TLR4 function in dermal fibroblasts.
Methods: Human fibroblasts were cultured at physiological glucose concentration (5.5 mM) and subsequently exposed to glucose concentrations from 0 mM to 25 mM for 24 hours. Identical samples were placed within a hypoxic chamber for 8 hours. Cell lysate protein and supernatant were harvested and western blot assays for TLR4, MyD88 and cleaved Caspase 3 were undertaken. Migration was assessed by scratch wound assay. The effects of MyD88 and TRIF inhibitory peptides and TLR4 neutralising-antibody and antagonist on fibroblast migration were assessed in the 25 mM glucose groups.
Results: Hypoxic conditions led to an increase in TLR4 protein expression. This effect was significantly increased ($p < 0.05$) in very high glucose concentrations (25 mM), and resulted in increased apoptosis (cleaved-caspase3) and IL-6 release. TLR4 inhibition reduced TLR4 protein expression and apoptosis ($p < 0.05$) in these same conditions. Hypoxia resulted in impaired fibroblast migration, particularly at high glucose concentrations ($p < 0.05$). Inhibition with a MyD88 inhibitory-peptide and a TLR4 neutralising-antibody and antagonist ameliorated the effects of high glucose and ischaemia ($p < 0.05$).
Conclusion: Hypoxia stimulates an up-regulation of TLR4 protein expression and this effect is exaggerated by hyperglycaemia. This results in an increase in cellular apoptosis. Inhibition of TLR4 resulted in a significant reduction in TLR4 protein expression and conferred a protective effect through reduced apoptosis. The migration of fibroblasts in hypoxia was also disproportionately impaired in the very high glucose treatment groups. TLR4 and myd88